VIA MEDICA

ORIGINAL ARTICLE

Folia Morphol.
Vol. 70, No. 3, pp. 161–167

ISSN 0015–5659
www.fm.viamedica.pl

Histomorphometric and sympathetic innervation of the human posterior intercostal artery and its clinical importance

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[Received 4 February 2011; Accepted 8 May 2011]

The purpose of this investigation was to study the characteristics of arterial wall and sympathetic innervation of the human posterior intercostal artery (PIA) in order to assess its suitability as an arterial graft for vascular surgeries. Fifty PIA samples were obtained from 25 cadavers (18 males and 7 females). Samples were divided into three age groups: group 1: 19–40 years; group 2: 41–60 years; and group 3 over 61 years. Sections (5 µm-thickness) of each sample were taken and stained with haematoxylin-eosin, Verhoeff's-Van Gieson. Five samples were processed for tyrosine hydroxylase immunostaining. The differences in the thickness of tunica intima were not statistically significant when group 1 was compared with group 2 ($p = 0.798$), but significant differences were observed in the thickness of the tunica intima when comparing group 2 with group 3 ($p = 0.012$) and group 3 with group 1 ($p = 0.002$). The tunica media was not statistically significant when group 1 was compared with group 2 ($p = 0.479$). However, significant differences were observed in the thickness of the tunica media when comparing group 2 with group 3 ($p = 0.001$) and group 3 with group 1 ($p = 0.011$). The mean (SD) number of elastic laminae in group 1, group 2, and group 3 were 7.88 ± 0.69, 6.62 ± 0.51, and 4.56 ± 0.82, respectively. Tunica intima/media ratios in groups 1, 2, and 3 were found to be 0.09 ± 0.01, 0.11 ± 0.02, and 0.27 ± 0.16, respectively. Tyrosine hydroxylase immunostaining revealed that sympathetic fibres are found mainly in the tunica adventitia and at the adventitia-medial border. The sympathetic nerve fibre area and sympathetic index were found to be 0.004 mm^2, and 0.151 mm^2, respectively. PIA has relatively thin intima and media, which are favourable features regarding its potential suitability as an alternate coronary by-pass conduit. (Folia Morphol 2011; 70, 3: 161–167)

Key words: sympathetic nerve fibres, ageing, elastic fibres, coronary by-pass graft

INTRODUCTION

Internal thoracic artery (ITA) is the standard arterial graft for the coronary artery by-pass surgery because of its long-term patency, favourable histological structure, the fact that it is less prone to pathological changes, and not least because of its anatomical position favouring its harvesting, both through sternotomy, thoracotomy, open surgery,

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Table 1. Distribution of human posterior intercostal artery samples

Groups	No. of male cadavers	No. of female cadavers	Total no. of cadavers	No. of arteries collected	
				Right	Left
Group 1 (19–40)	7	2	9	9	9
Group 2 (41–60)	5	3	8	8	8
Group 3 (≥ 61)	6	2	8	8	8
Total	18	7	25	25	25

minimally invasive surgery, or thoracoscopy [3, 18, 22]. In the last few decades several other arteries have been introduced as reliable alternative arterial conduits, including the radial artery [1, 2], inferior epigastric artery [4], gastroepiploic artery [14, 20], and splenic artery [15], to accomplish total arterial myocardial revascularisation.

The posterior intercostal artery (PIA) has been proposed as an alternative arterial conduit in a study that demonstrated its favourable histological characteristics [21, 23]. A human cadaveric study showed that in situ PIA grafts to the major coronary artery territories are anatomically feasible and offer the possibility of an alternative arterial conduit [13]. Dandolu et al. [5] have harvested 8th and 9th intercostal arteries as pedicle grafts in 12 dogs, initially by thoracic approach and then via median sternotomy. These pedicle samples were able to reach both branches of the right and left coronary arteries.

One of the complications observed after the bypass surgeries is vasospasm of the graft. Knowledge of the mechanism of how vasospasm develops is still lacking. However, it is presumed that vasospasm is the extreme form of vasoconstriction, which may be associated with the response of a vessel to perivascular nerves and composition of arterial wall. Hence, we proposed to study the characteristics of the arterial wall structure and sympathetic nerve supply of the human PIA.

MATERIAL AND METHODS

Sample collection and fixation

Fifty bilateral PIAs were dissected during autopsy from 25 cadavers (18 males and 7 females) who died of non-cardiovascular diseases. The cadavers were aged between 19 and 83 years. All arterial samples were divided into 3 groups according to age: Group 1, samples of those aged 19–40 years; Group 2, samples of those aged 41–60 years; and Group 3, samples of those aged over 61 years. The distribution of PIA samples is shown in Table 1. All the samples were immediately fixed with 4% paraformaldehyde for 24 hours and subsequently processed for histological methods without any delay. All the samples were processed with haematoxylin-eosin (H&E), Verhoeff-Van Gieson (VVG) stains for histopathological and histomorphometric studies. Five out of fifty arteries were processed for the tyrosine hydroxylase (TH) immunostaining.

Method of collection

Samples were collected according to the procedure used by van Son et al. [23]. Using a Rokitansky incision, the heart and lungs were removed from the thoracic cavity of the cadavers. The PIA was identified and about 2 cm length of the artery was obtained from the 5th intercostal space, approximately 4 cm from its origin (from the thoracic aorta).

Tissue processing for histological methods

Samples were dehydrated in 50%, 70%, 90%, and absolute alcohol, cleared in xylene, impregnated with paraffin, and then embedded in paraffin. Five-micron sections were taken with rotary microtome and mounted on gelatine-coated slides and stained with H&E and VVG.

Tissue processing for immunohistochemistry

Paraformaldehyde fixed samples were cryoprotected in phosphate buffer saline (PBS) containing 20% sucrose for 24 hours and then mounted with tissue freezing medium. Five-μm sections were taken by using a Leitz cryostat at –20° C and collected onto APES (3-aminopropyl triethoxysilane) coated slides.

Tyrosine hydroxylase immunostaining

Sections were washed in PBS (2 × 5 min), treated with peroxidase block for 30 minutes, and then washed in PBS (2 × 5 min). Subsequently, the sec-

Table 2. Descriptive statistics of thickness of Ti of human posterior intercostal artery

Groups	No. of samples	Mean ± SD [μm]	95% CI		P-value
			Lower bound	Upper bound	
Group 1 (19–40)	18	6.77 ± 0.87	6.1	7.44	G1–G2 = 0.798
Group 2 (41–60)	16	8.21 ± 0.97	7.4	9.03	G2–G3 = 0.012
Group 3 (≥ 61)	16	15.5 ± 8.06	8.75	22.24	G3–G1 = 0.002
Total	50	10.03 ± 5.87	7.6	12.45	

The differences in the thickness of Ti were not statistically significant when G1 was compared with G2. Note that significant difference observed in thickness of Ti when comparing G2 with G3 and G3 with G1 (oneway ANOVA followed by Tukey HSD post hoc test)

tions were blocked with normal goat serum for 1 hour, followed by incubation in rabbit polyclonal anti-TH primary antibody (AB152, Millipore, Temecula, CA, USA) diluted 1:100 in PBS for 48 hours at 4°C. Sections were washed in PBS (2 × 5 min), incubated in biotinylated goat anti rabbit secondary antibody (Sc-2051, Santa Cruz, CA, USA) for 2 hours followed by incubation in HRP-streptavidin (Sc-2051, Santa Cruz, CA, USA) complex for two hours. Finally, colour was developed by treating the sections with DAB (Sc-2051, Santa Cruz, CA, and USA) for 5 minutes. The sections were then washed with distilled water, counterstained with haematoxylin, dehydrated with two changes of alcohol, cleared in xylene, and cover-slipped.

Human adrenal glands were used as positive controls and processed as above at the same time. For the negative control, sections were incubated in normal goat serum replacing primary antibody.

Analysed parameters

Stained sections were observed under binocular light microscope, and digital images were obtained. The digital images were analysed for the following histomorphometric parameters:

1. Thickness of tunica intima (Ti) and tunica media (Tm) were measured by using Leica Qwin V3 software at a magnification of 400×. Thickness of Ti and Tm were measured at five random places and then means were obtained.
2. Number of elastic laminae (Nel) was obtained at a magnification of 400×.
3. Adventitial area and sympathetic nerve fibre content was obtained at a magnification of 100× by using in-house developed software named "Tissue Quant", which is designed for colour quantification. This software provides the facility to choose a colour for selectively choosing the pixels in the image with the chosen colours and its shades. For the purpose of calibration, images of scales both in horizontal and vertical positions were obtained under the same magnification for the calibration purpose. The number of pixels representing a length of 1 mm was calculated for both horizontal and vertical arrangements. This provided the calibration for the number of pixels representing one mm^2 of area.

Statistical analysis

Statistical analysis was performed using SPSS 11.5 software. Data were expressed as mean ± standard deviation (SD) and 95% confidence interval (CI). Data were analysed by one way ANOVA followed by Tukey HSD post-hoc test. Probability (P) values less than 0.05 were considered significant.

RESULTS

Histomorphometric results

The mean, SD, 95% CI (lower bound and upper bound), and p values of thickness of Ti in Group 1 (G1), Group 2 (G2), and Group 3 (G3) are depicted in Table 2. The thickness of Ti in G1, G2, and G3 were 6.77 ± 0.87 μm, 8.21 ± 0.97 μm, and 15.5 ± ± 8.06 μm, respectively. The differences in the thickness of Ti were not significant when comparing G1 with G2 (p = 0.798), but there were significant difference observed when comparing G2 with G3 (p = 0.012) and G3 with G1 (p = 0.002).

The mean, SD, 95% CI (lower bound and upper bound), and p values of Tm in G1, G2, and G3 are presented in Table 3. Mean thickness of Tm of PIA in G1, G2, and G3 were 71.55 ± 10.11 μm, 75.80 ± 4.68 μm, and 59.97 ± 5.94 μm, respectively. The thickness of Tm was statically significant when comparing G2 with G3 (p = 0.001) and G3 with G1 (p = 0.011). However, there were no statistically significant differences observed when comparing G1 with G2 (p = 0.479).

Tunica intima/media ratios (Ti/Tm) of PIA for each age group in the study are depicted in Table 4. It

Table 3. Descriptive statistics of thickness of Tm of human posterior intercostal artery

Groups	No. of samples	Mean ± SD [μm]	95% CI		P-value
			Lower bound	Upper bound	
Group 1 (19–40)	18	71.55 ± 10.11	63.77	79.32	G1–G2 = 0.479
Group 2 (41–60)	16	75.8 ± 4.68	71.88	79.72	G2–G3 = 0.001
Group 3 (≥ 61)	16	59.97 ± 5.94	55	64.94	G3–G1 = 0.011
Total	50	69.2 ± 9.78	65.16	73.24	

Tm was not statistically significant, when G1 was compared with G2. However, significant difference was observed in thickness of Tm when comparing G2 with G3 and G3 with G1 (oneway ANOVA followed by Tukey HSD post hoc test)

Table 4. Descriptive statistics of Ti/Tm ratios of human posterior intercostal artery

Groups	No. of samples	Mean ± SD	P-value
Group 1 (19–40)	18	0.09 ± 0.01	G1–G2 ≥ 0.05
Group 2 (41–60)	16	0.11 ± 0.02	G2–G3 ≤ 0.001
Group 3 (≥ 60)	16	0.27 ± 0.16	G3–G1 ≤ 0.001
Total	50	0.16 ± 0.10	

Tunica intima/media ratio (Ti/Tm) was not statistically significant, when G1 was compared with G2. However, significant difference was observed in Ti/Tm ratio when comparing G2 with G3 and G3 with G1 (oneway ANOVA followed by Tukey HSD post hoc test)

Table 5. Descriptive statistics of number of elastic lamina of human posterior intercostal artery

Groups	No. of samples	Mean ± SD	95% CI		P-value
		Lower bound	Upper bound		
Group 1 (19–40)	18	7.88 ± 0.69	7.35	8.42	G1–G2 = 0.003
Group 2 (41–60)	16	6.62 ± 0.51	6.19	7.05	G2–G3 ≤ 0.0001
Group 3 (≥ 60)	16	4.56 ± 0.82	3.87	5.24	G3–G1 ≤ 0.0001
Total	50	6.42 ± 1.55	5.77	7.06	

Number of elastic laminae was found to have decreased with age. Note that there are statistically significant differences in Nel, when comparing G1 with G2, G2 with G3 and G3 with G1 (oneway ANOVA followed by Tukey HSD post hoc test)

Table 6. Adventitial and sympathetic nerve fibre areas of posterior intercostal artery

S. no	Age	Sex	Side	Ada [mm^2]	Sympa [mm^2]	SI
1	19	F	Right	0.025	0.003	0.135
2	41	M	Right	0.020	0.003	0.138
3	47	M	Left	0.026	0.004	0.133
4	63	M	Right	0.025	0.005	0.194
5	77	M	Left	0.024	0.004	0.157
Mean				0.024	0.004	0.151

Sympathetic index (SI) to posterior intercostal artery was calculated by dividing the sympathetic fiber area by the adventitial area; Ada — adventitial area; Sympa — sympathetic area

was found that the Ti/Tm ratio increased with age. Ti/Tm ratios were found to be significant when G2 compared with G3 ($p \leq 0.001$) and G3 with G1 ($p \leq 0.001$). However, there were no statistically significant differences observed when comparing G1 with G2 ($p > 0.05$).

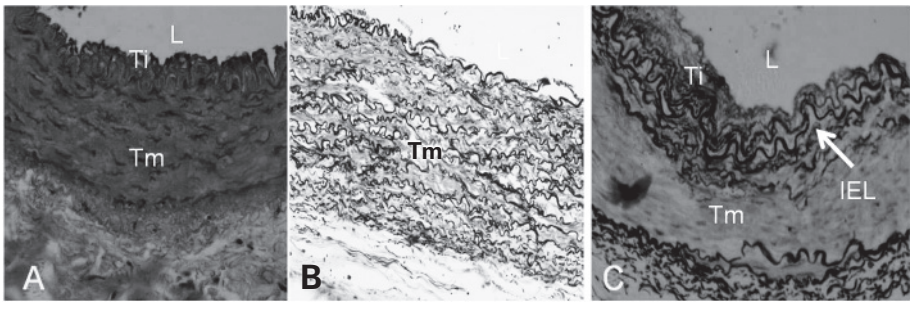

Figure 1. A. Photomicrograph of posterior intercostal artery (PIA) wall of group 1 sample (32 years) stained with H&E, showing no age--related pathological changes (400×); **B.** PIA of group 2 sample (55 years) stained with Verhoeff-Van Gieson stain, showing numerous elastic laminae in the tunica media (Tm) (400×); **C.** PIA of group 3 sample (83 years) showing decreased number of elastic laminae in the Tm, mild intimal thickening, and fragmented IEL (400×); L — lumen; Ti — tunica intima; IEL — internal elastic lamina.

The mean number of elastic laminae in G1, G2, and G3 were 7.88 ± 0.69, 6.62 ± 0.51 and 4.56 ± ± 0.82, respectively (Table 5). Regarding the number of elastic laminae, there were significant differences observed when comparing G1 with G2 ($p = = 0.003$), G2 with G3 ($p \leq 0.0001$), and G3 with G1 ($p \leq 0.0001$).

The findings of the present study suggest that the thickness of Ti and the Ti/Tm ratio were increased, whereas the number of elastic laminae and the thickness of Tm was found to have decreased in relation to age.

Histological study suggests that age-related pathological changes like intimal thickening or atherosclerosis are not observed in the PIA samples of G1 and G2; however, mild intimal thickening was found in 8 out of 16 samples in G3. (Fig. 1). Calcification of Tm was not found in any of the PIA samples studied. Tm showed few smooth muscles and numerous elastic laminae, which were arranged concentrically (Fig. 1B). The internal elastic lamina (IEL) and external elastic lamina (EEL) were well developed and continuous in all samples of G1 and G2, while discontinuations of the IEL were observed in the samples from G3 (Fig. 1C).

Immunohistochemistry results

TH immunostaining revealed that sympathetic nerve fibres were seen in the tunica adventitia and at the adventitia-media border (Fig. 2). The adventitial and sympathetic nerve fibre areas of PIA are shown in Table 6. The mean adventitial and sympathetic nerve fibre areas were found to be 0.024 mm^2 and 0.004 mm^2, respectively. The sympathetic index (SI) to PIA was calculated by dividing the sympathetic nerve fibre area by the adventitial area. The mean SI value was found to be 0.151.

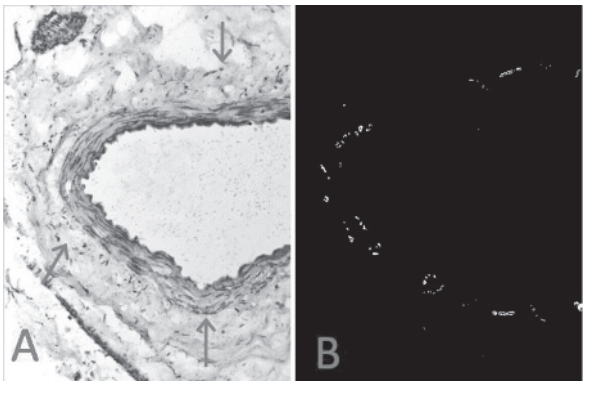

Figure 2. A. Arrows pointing to the sympathetic fibres in a posterior intercostal artery (PIA) of a 47-year-old individual stained with TH immunostaining (100×); **B.** Results of the automated measurement of sympathetic fibre area (white dots) of the same PIA that was calculated by Tissue Quant image analysis software (100×); 1 mm^2 sympathetic fibres area = approximately 2540 × 2450 pixels.

DISCUSSION

In the present study, PIA showed the structure of an elastic artery. Tunica media of PIA had concentrically arranged elastic laminae. The highest number of elastic laminae was seen in group 1 samples and the lowest in group 3 samples. The number of elastic laminae in the Tm decreased with age. Van Son et al. [23] studied the structure of the PIA in cadavers and found three combinations of histological patterns along the course of the intercostal artery: a proximal elastic segment followed by subsequent elastomuscular and muscular segments. In the present study, the proximal part of PIA was harvested, which was composed mainly of elastic laminae with very few smooth muscles and was similar to a van Son et al. [23] type I pattern. Comparative histology of ITA versus other arteries

such as coronary, radial, ulnar, epigastric, and right gastroepiploic arteries were studied by several researchers, who proposed that the internal thoracic is an elastic artery and the others are muscular arteries not prone to pathological changes when compared with other arterial conduits [1, 3, 14, 21].

Unlü et al. [21] reported that the histological structure of the PIA is similar to the structure of the ITA. Both these arteries are elastic and not prone to atherosclerosis. This may be because these conduits have elastic laminae, perfect continuity of IEL (except in the samples of seventh and eighth decades of life), and EEL and are resistant to atherosclerosis.

In the present study, the tunica intima of PIA was thin and well developed in all the samples studied except in the G3 samples, which showed mild intimal hyperplasia in the seventh and eighth decades of life. This may be associated with fragmentations in the IEL during the ageing process. The IEL and elastic laminae in the Tm play an important role in the prevention of intimal thickening because they form a barrier to the invasion of smooth muscles cells from the Tm into the Ti [18]. Discontinuity of the IEL might cause migration of smooth muscle cells from media to intima and might activate atherosclerosis [18].

The mean thickness of Tm in groups 1, 2, and 3 were 71.55 μm, 75.8 μm, and 59.97 μm, respectively. The present study revealed that the thickness of Tm decreased with age. It can be suggested that this is be due to the fact that the number of elastic laminae may decrease as age advances. Furthermore, the present study has demonstrated that the PIA has a relatively thin Tm that may be advantageous with regard to its potentially lower tendency toward intimal hyperplasia and ischaemia of the media. Previous studies have shown an increased tendency of intimal hyperplasia and ischaemia of the media in the muscular artery conduits with a thick media (radial artery, inferior epigastric artery, gastroepiploic artery, and splenic artery) [1, 2, 4, 14, 15, 20].

In the present study Ti/Tm ratios of PIA for each age group were studied, and it was found that the Ti/Tm ratio increased with age. This may be attributed to the intimal changes in reaction to pressure and blood flow dynamics. Previous studies have shown intimal thickness and an age-dependent increases in Ti/Tm ratios in the coronary arteries, intracranial and extraparenchymal cerebral arteries, and abdominal organ arteries [12, 16, 24, 25]. The aorta also demonstrated age-dependent as well as site-dependent increases of Ti/Tm ratio [16]. The mechanism of this age-related change is not known but may be related to body size because intimal thickening is not present in small mammals, such as the mouse and rat, but is present in larger mammals like swine and horse [8, 17].

The advantageous features of the PIA regarding its potential suitability as a conduit in myocardial revascularisation are:

— PIA grafts were able to reach any of the major coronary artery territories [5, 13];
— the presence of a thin tunica intima, media, and multiple elastic lamellae as observed in this study;
— its close proximity to the heart, and the fact that it is easily dispensable and readily available [23].

Immunohistochemical study revealed that sympathetic nerve fibres were found and were situated mainly in the tunica adventitia and at the adventitia-media border. There have been limited studies that have quantified the sympathetic fibre content in the arterial grafts. Gaudino et al. [9] and Deja et al. [6] found sympathetic nerve fibres in adventitia of ITA by using TH and S-100 immunostaining, respectively. Barry et al. [3] showed sympathetic and parasympathetic fibres in the adventitia of radial, ulnar, epigastric, and coronary arteries. Sympathetic fibres could be the cause for spasm of arterial grafts. Knowledge of how vasospasm develops is still lacking. However, it is presumed that vasospasm is an extreme form of vasoconstriction, which may be the response of a vessel to many stimuli, such as: physical (mechanical stimulation or temperature changes) or pharmacological (nerve stimulation or vasoconstrictor substances) [10]. According to Suma [19] and Fisk et al. [7], the tendency to vasospasm is higher in the gastroepiploic and radial arteries than in the ITA. Functionally, arteries have been classified into three types: (1) type I (somatic), (2) type II (splanchnic), and (3) type III (limb arteries). Types II and III arteries (muscular arteries) were reported to be more prone to spasm than somatic arteries such as the ITA and PIA [11, 10]. In the present study, SI was assigned to PIA. The mean SI value was 0.151. Sympathetic index may be used to correlate and compare sympathetic fibre related problems of the PIA.

CONCLUSIONS

It can be suggested that PIA is an elastic artery although anatomically it is considered as medium-sized artery. Mild intimal hyperplasia or atherosclerosis was observed in elderly cases with no medial calcification. Thicknesses of Tm and number of elastic laminae were

found to have decrease as age advanced. Tunica intima and media ratio increased with age. SI index may be used for sympathetic nerve fibre related problems of PIA. PIA may be considered as an alternate conduit in myocardial revascularisation.

ACKNOWLEDGEMENTS

We would like to sincerely thank Manipal University for providing the experimental facilities to carry out this work. We would also like to thank Dr. Jerry George Mathew for proofreading the manuscript.

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